Research Article

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Essential oil yield and composition of *Pistacia vera* 'Kerman' fruits, peduncles and leaves grown in California

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Abstract

BACKGROUND: *Pistacia vera* 'Kerman' is the predominant pistachio nut cultivar in the United States (California), the world's second largest producer. Despite several reports on the essential oil (EO) content in the genus *Pistacia*, data on 'Kerman' are limited. The EO content and volatile organic compound (VOC) emissions of tree nut orchards are of current interest to researchers investigating insect pests and the potential role of EO and VOCs as semiochemicals. To establish a basis for the VOC output of pistachios, the EO content of fruits, peduncles, and leaves was analyzed.

RESULTS: Evaluated plant parts contained limonene as the primary EO component, followed by α-terpinolene. Peduncles were unique in containing relatively high levels of α-thujene. The results were reproducible between two different geographical locations. *In situ* solid phase microextraction (SPME) studies demonstrated the volatile emission was representative of the EO composition.

CONCLUSION: This is the first report detailing the content and distribution of EO and the unique limonene-dominant profile for this *Pistacia vera* cultivar which may influence pistachio insect pest semiochemical research.

Keywords: essential oil; Kerman; limonene; peduncles; *Pistacia vera*; terpinolene; thujene

INTRODUCTION

Pistacia vera L. (Anacardiaceae), commonly known as pistachio, is an agricultural product cultivated in semi-arid regions worldwide.[1] Iran is the major cultivator, producing approximately 230 000 metric tons annually,[2] followed by the United States, which produced 139 000 metric tons in 2008 and generated an annual revenue of more than $539 million.[3] Pistachios, along with almonds and walnuts, are major tree nut crops in California with combined annual revenue of over $3.3 billion. Approximately 50–70% of California tree nuts are exported overseas annually and are subject to strict export action levels.[4]

Commercial production of pistachios in California is effectively based on one cultivar, Kerman. Dependence on a singular genetic line has been shown to render a crop vulnerable to new diseases as well as native insect pests.[1] However, efforts are under way to diversify and introduce a range of other cultivars. The control and monitoring of tree nut insect pests are priorities of the California tree nut industry.[5] Insect pests such as navel orangeworm,[6] redshouldered stink bug, flat green stink bug, leaffooted bug, and *Calocoris* spp. cause considerable monetary losses to growers and processors.[7] Feeding damage lowers kernel quality, thus decreasing the value, and moreover, indirect damage is done by the associated contamination of toxin-producing fungi, mycotoxins.[8]

Insect pests are known to use cues from plant volatile organic compounds (VOCs) for host-plant recognition, which includes the choice, acceptance or rejection of a plant part for feeding, oviposition, and/or larval development. These VOC cues, commonly referred to as plant semiochemicals, have also been implicated in the modulation of pheromonal activity.[9,10] Additionally, the tree nut insect pest, navel orangeworm, shows electrophysiological responses to ambient orchard VOCs.[11]

Monoterpenes stored as essential oil (EO) typically correlate with the monoterpene emissions.[12] Like other related species in the genus, *P. vera* is known to accumulate EO in its leaves and fruit hulls;[13–16] however, reports on the EO content in stems of the fruit clusters, known as peduncles, are not available.

Of crops occupying large acreages in California's Central Valley, *P. vera* cv. Kerman has been identified as a major emitter of VOCs. 'Kerman' pistachio trees emit 12 $\mu g\ h^{-1}\ g^{-1}$ of VOCs, primarily monoterpenes;[17] yet the monoterpene composition and the proportional contribution of different plant parts are unclear. While several reports on EO content are available for other species in the genus *Pistacia*, data on the EO content and composition of *P. vera*, and 'Kerman' in particular, are scarce.

The aim of the present study was to establish the organ-specific EO concentrations and relative proportions of the major EO components from 'Kerman' grown in California's Central Valley. Measurement at a single developmental stage, just prior to harvest,

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provides a basis for comparison to other cultivars and complement data with ongoing ambient orchard VOC research.

MATERIALS AND METHODS

Plant material

Plant samples were collected from Paramount Farming Company orchards located near Lost Hills, CA, in the southern part of the Central Valley on 2 September 2009 (3098 degree days, biofix Jan 1, NOW), and from Strain Ranches near Arbuckle, CA, in the northern part of the Central Valley on 16 September 2009 (3007 degree days). The female *Pistacia vera* cv. Kerman trees in the orchards investigated bear five to seven leaves at the apex of each branch followed proximal by panicles with green ramified peduncles, typically three to five in number. Aside from the apical leaves and panicles the fruiting branch is usually free from any leaves or panicles and is lignified. The fruiting branch tip is therefore the repetitive element of the tree and was used for the present analyses as a representative sample for each tree. Fruiting branch tips ($n = 10$) were collected between 11:00 am and 3:00 pm. Each branch was collected from a different tree and no more than three branches per irrigated row. A total of 10 branches were combined to give one composite sample. The samples were transported (2–5 h) in paper bags to the laboratory at approx. 20 °C, and stored in sealed plastic bags at 5 °C until processing. The different plant parts were separated using a pruning tool. The hydrodistillation was performed either immediately after separation of the plant parts (peduncles) or after storage at 5 °C within the following 24 h. Peduncles were hydrodistilled first because they produce exudate 'bleeding' upon injury, but leaves and fruits were hydrodistilled in varying order.

Processing and hydrodistillation

The plant material was processed while wearing nitrile gloves in the following manner to minimize cross-contamination of plant parts with the sap exudate from peduncles. The branches were held by the woody stem portion, and first the leafy tip was removed and placed separately (non-bleeding). The fruits were then cut off the ramified peduncle using a pruning tool. Less than 1 mm of the pedicel remained on the fruit. Next, the peduncles were cut off the woody portion of the stems. Any damaged portions were excluded. Leaves and peduncles were cut into pieces of size 2–5 cm and immediately placed in a flask that contained sufficient water for the plant material to be completely immersed.

Hydrodistillation proceeded with a typical Clevenger-type set-up[18] for essential oils lighter than water using deionized water (≥18.0 MΩ cm). The freshly cut plant material was immediately immersed in the water and the level filled to about half the flask volume. The collection was terminated when no increase in essential oil level was detectable for longer than 1 h, with total distillation times between 2 and 3.5 h from the time condensation was first observed.

After collection, the EO was carefully taken up in pentane, dried over anhydrous sodium sulfate, diluted to a total volume of 5 mL, and stored in a Teflon-capped amber vial at 4 °C until analysis.

GC–MS analysis of essential oil

Separation and identification of the collected EO mixture was achieved via standard methods used in this laboratory.[11] Instrumentation was as follows: J&W Scientific (Folsom, CA, USA) DB-Wax column (60 m × 0.32 mm i.d. × 0.25 μm), installed on an HP-6890 gas chromatograph (GC; Agilent, Santa Clara, CA, USA) coupled to an HP-5973 mass selective detector (MS; Agilent). Diluted EO components were analyzed with the following method: inlet temperature, 200 °C; pressure, 8.25 psi; total flow, 34.1 mL min^{-1}; split mode, 20:1; constant He flow, 1.5 mL min^{-1}; velocity, 31 cm s^{-1}; oven settings, initial temperature, 40 °C; hold time, 0.0 min; ramp one, 4 °C min^{-1}; final temperature, 200 °C; hold time, 15 min; post-run 220 °C for 5 min. NIST, Wiley, and internally generated databases were used for fragmentation pattern identification. All major EO components, except for myrcene, were verified by injection of authentic standards and comparison of retention times. Additionally, the retention indices (RIs) were calculated using a homologous series of *n*-alkanes on a DB-Wax and DB-1 column using literature GC–MS parameters.[11]

SPME GC–MS analysis of *in situ* and EtOH VOCs

The *in situ* VOCs were collected using a Teflon bag and SPME in a manner identical to the published method by Beck *et al*,[11,19] and following 'P.E.S.T' (permeation, exposure, storage, thermal desorption).[19] Permeation of VOCs in collection bag, P = 5 min; exposure of SPME fiber to VOCs, E = 90 min; storage of VOCs on SPME fiber, S = 24 h (on ice); and, thermal desorption of VOCs onto GC injector, T = 5 min. The *in situ* VOCs were collected at 1:45 to 3:40 pm from the Arbuckle, CA, site on 16 September 2009. The VOCs of the EtOH headspace were collected with the following parameters: P = 0 min; E = 1 min; S = 5 min; T = 5 min.

RESULTS AND DISCUSSION

Hydrodistillation of each plant part afforded varying amounts of colorless essential oil lighter than water, decreasing in order from peduncles with 0.45 mL, fruits with 0.25 mL, and finally leaves with 0.1 mL (Table 1). Considering the individual fresh weights, which related as about 1:10:1, the calculated EO concentrations were 3.1, 0.17 and 0.5 mL kg^{-1}, for peduncles, fruits and leaves, respectively, identifying peduncles as the major essential oil bearing plant part in 'Kerman'.

A previous study[16] using a comparatively small sample (100 g) of *P. vera* from Greece found higher levels of essential oil in fresh young fruits than in leaves, a finding contrary to the present study in which mature fruits were investigated. In the present study, the EO concentration found in the leaves was less than half of that previously reported for *P. vera* (0.1% and 0.15%) from southern Europe.[13,16] This difference may be due to cultivar differences in the genetic potential to accumulate essential oil. Alternatively, the present mid-day/early afternoon collection may show reduced leaf essential oil content due to partial evaporation over the course of the day. Definitive comparison is untenable, however, since cultivar and time of collection were not specified previously. The EO content of unripe fruit collected in May in Greece has been reported to be 0.5% w/w.[16]

The major components (>1% relative peak area) in all EO samples were familiar monoterpenes (Table 2). Limonene was the dominant component in each plant part, with 80–85% in fruit EO and 78–81% in leaf EO. The lowest limonene levels (50–52%) were found in peduncle EO. The very high limonene levels for this cultivar are in agreement with a previous report which indicated that the major constituent of 'Kerman' leaf

Table 1. Essential oil yield of 10 fruiting branches and concentration in different plant organs of *Pistacia vera* cv. Kerman

		Peduncles		Fruits		Leaves	
Sample[a]	Orchard location (county)	Yield[b] (mL)	Conc. (mL kg^{-1})	Yield (mL)	Conc. (mL kg^{-1})	Yield (mL)	Conc. (mL kg^{-1})
1	Kern	0.4	2.8	0.2	0.16	0.1	0.6
2	Kern	0.4	2.6	0.3	0.19	0.1	0.7
3	Colusa	0.5	3.7	0.3	0.20	0.1	0.5
4	Colusa	0.5	3.4	0.2	0.13	0.1	0.4
Mean		0.45	3.1	0.25	0.17	0.1	0.5

[a] Each sample is composed of 10 fruiting branches from different trees and rows, see Materials and Methods section for details.
[b] Accuracy is ±0.05. Conc. = concentration, based on fresh weight.

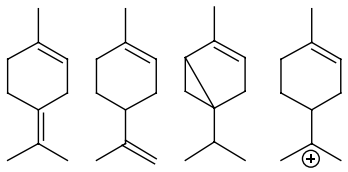

Figure 1. Major essential oil components of 'Kerman': α-terpinolene (**1**), limonene (**2**), α-thujene (**3**), and their common biogenic cation intermediate (**4**).

volatiles is limonene, constituting 46% of the headspace volatiles collected from fresh leaves.[20] These findings are, however, in stark contrast to results from unripe *P. vera* fruits from Greece,[16] which contained 54.6% α-pinene and 31.2% α-terpinolene, as major components, and only 2.5% limonene in the EO. In the same report, the EO from leaves contained 30.0% α-pinene, 17.6% α-terpinolene, and only 3% limonene. In another report,[15] the EO of hulls contained 54.4% α-pinene, and in yet another study[13] the EO of leaves contained 29.2% α-pinene as the major component, not limonene.

The closely related isomer, α-terpinolene (Fig. 1), was generally the second highest compound with 6–10% in fruit EO and 9–10% in leaf EO; other compounds were only minor. Terpinolene was also the second highest compound in peduncle EO, in most instances with an average of 14% at higher level relative to limonene. Perhaps the most strikingly different EO of the different plant parts was that of the peduncle, which was more complex and in which terpinolene was closely followed by α-thujene (13%, Fig. 1) in abundance. Fruits and leaves were virtually devoid of α-thujene (<1%). Aside from limonene, α-terpinolene, and α-thujene, all other components were relatively minor, except for α-pinene (Table 2).

Interestingly, the three major EO components, compounds **1, 2** and **3**, shown in Fig. 1, are monoterpenes with closely related biogenesis from the common cation intermediate, **4**. Minor compounds that were above the 1% threshold for this study include five related monoterpenes: bornyl acetate, camphene, Δ^3-carene, myrcene, and α-pinene. It should be noted that only a small number of related enzymes could produce these profiles, since monoterpene synthases are known to form multiple products.[21]

The EO content of *P. vera* was evaluated from two different geographical regions of California, southern Central Valley and northern Central Valley. The two locations are more than 450 km apart. To minimize EO differences due to phenological development, the plant parts from both locations were collected at the beginning of split. The relative EO composition and yields between different regions were surprisingly similar, contrary to studies that have shown that different environments, such as climate, soil and water supply, may influence the EO composition and yield of a single cultivar.[22,23] However, if the relative climatic conditions between the two locales are taken into consideration: Lost Hills : Arbuckle; sunny days, 274 : 265; average July high temperature (°C), 38.2 : 35.9; elevation (m), 132 : 39; and rainfall (cm), 8.1 : 36.6, it is plausible that the differences are discrete enough to allow for similar EO composition profiles. However, a more in-depth study would have to be undertaken to definitively determine geographical and/or climate differences.

Limonene is a semiochemical with known broad attributes of attractant, kairomone, allomone and pheromone. While the specific enantiomer was not identified for the isolated/identified compound, both enantiomers, as well as the general structure, have demonstrated semiochemical behavior.[24] In a study from Turkey, *P. vera* leaves and fruit contained a mixture of enantiomers in a D/L ratio of 80:20.[13]

The EO component α-terpinolene possesses broad reported characteristics as an allomone, attractant and kairomone, primarily among Isoptera and Coleoptera, among others. Additionally, α-terpinolene is a ubiquitous VOC found in several flowering plants, largely in the family Orchidaceae.

The compound most differentially accumulated, α-thujene, has the least number of identified semiochemical activities. However, it is reported as an aphid (Homoptera) pheromone and as a termite (Isoptera) allomone. Since it is accumulated to high levels in peduncles, it may have an unidentified biological function for the plant, either alone or in mixtures.

In situ SPME studies on bagged intact branch tips confirmed that all major constituents in Table 2 were emitted into the immediate surrounding atmosphere. When fruits were directly collected in the field and placed immediately into 95% EtOH to inhibit any enzyme activity, the headspace VOCs of the EtOH extract showed a very similar limonene dominant profile, verifying it is not due to postharvest changes during transport and storage. All VOCs reported in Table 2 were also seen in the EtOH extract headspace analysis.

CONCLUSION

The major female cultivar of *Pistacia vera*, Kerman, grown in California was found to contain varying amounts of essential oil in its non-lignified portions of the branches. The EO of each of these plant parts contains limonene as a major component, which differs

Table 2. Major essential oil components of different plant organs of Pistacia vera cv. Kerman[a]

	DB-1 RI		DB-Wax RI		Colusa			Kern		
Compound	Lit[b]	Calcd[c]	Lit[b]	Calcd[c]	P	F	L	P	F	L
Limonene	1020	1025	1197	1200	+++	++++	++++	+++	++++	++++
α-Terpinolene	1077	1081	1278	1281	++	++	++	++	++	++
α-Pinene	929	934	1020	1019	++	+	+	++	+	+
α-Thujene	922	927	1022	1023	++	tr	tr	++	tr	tr
Δ-3-Carene	1004	1005	1144	1145	+	+	+	+	+	+
Myrcene	981	985	1157	1161	+	+	+	+	+	+
Bornyl acetate	1268	1270	1578	1578	+	tr	+	+	tr	+
Camphene	941	946	1063	1061	+	tr	tr	+	tr	+
β-Pinene	968	972	1106	1105	+	tr	tr	+	tr	tr

[a] Volatile relative peak area percent: tr = trace, <1;
+ = 1–5;
++ = 5–20;
+++ = 20–60;
++++ = 60–90; average of two independent measurements in the same orchard, 10 branch tips (trees) combined for each measurement; P = peduncles, F = fruit, L = leaf; compound identification by RI relative to *n*-alkanes on DB-1 and DB-Wax columns, retention times, mass fragment libraries, and comparison to authentic samples.
[b] Compound RI of literature value.
[c] Calculated compound RI based upon retention time on respective column.

from previous reports for this species where limonene was present only at relatively low levels. The peduncles of 'Kerman' are identified as the major EO bearing plant part. The composition of this EO is distinct in that it contains α-thujene as the third major component, the leaf and fruit EOs are nearly devoid of this compound. These results are to be understood as a phenological snapshot and provide a point for comparison for further studies into potential diurnal fluctuations, seasonal variation, VOC emissions, and organ-specific phytochemical comparisons of new cultivars in California.

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